7-DEAZAGUANOSINE: PHOSPHORAMIDITE AND PHOSPHONATE BUILDING BLOCKS FOR SOLID-PHASE OLIGORIBONUCLEOTIDE SYNTHESIS

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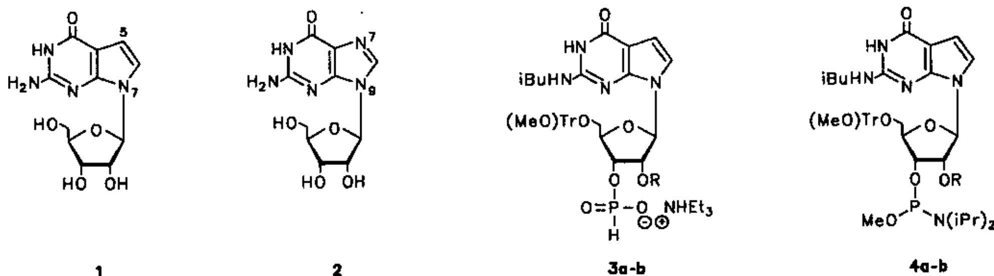
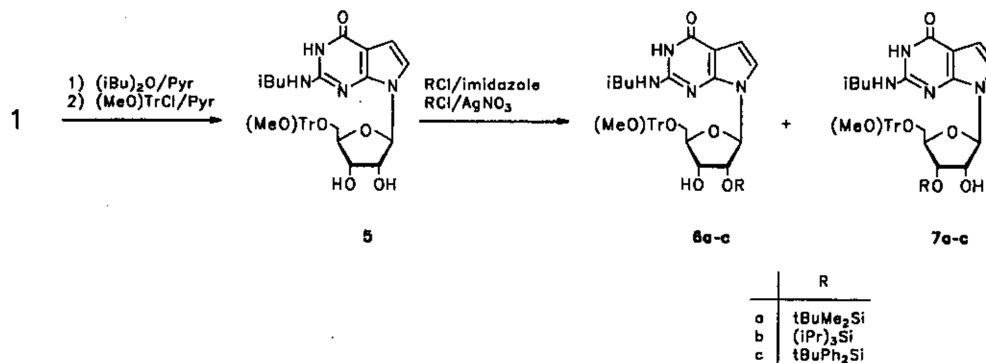
Abstract——The synthesis of oligoribonucleotide building blocks (phosphonates and phosphoramidites) of 7-deazaguanosine (2-amino-7-(ß-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one, **1**) is described. Silylation of the 2'-hydroxy group of 7-deaza-N^2-isobutyryl-5'-*O*-(4-monomethoxytrityl)guanosine (**5**) was studied with regard to the 2'-selectivity. Best results were obtained with triisopropylsilyl chloride/$AgNO_3$. The 2'-*O*-protected intermediates (**6a** and **6b**) were converted into the phosphonates (**3a**, **b**) or methylphosphoramidites (**4a**, **b**), which were successfully used for solid-phase oligoribonucleotide synthesis of $(c^7G\text{-}C)_3$ (**9**).

Guanine-rich regions of nucleic acids can associate to form multi-stranded structures. Enzymatically prepared poly(G) can aggregate to four-stranded helices.[1] Tetramerization of short tracts of G-residues in naturally occurring RNA has also been reported.[2] In the case of 2'-deoxyguanosine containing nucleic acids, so-called G4-DNA[3] is formed.

Earlier investigations have shown that N-7 participates in hydrogen bonding of such structures.[4] As a consequence, we have reported on oligodeoxyribonucleotide building blocks of 7-deaza-2'-deoxyguanosine for solid-phase synthesis[5] as well as di- and triphosphates[6,7] for enzymatic polymerization. In the following we present the synthesis of phosphoramidites and phosphonates of suitably protected 7-deazaguanosine (c^7G, **1**) and their use in solid-phase oligoribonucleotide synthesis.



The starting material, compound (**1**), was synthesized as described earlier.[8,9] For the protection of the exocyclic amino group, the isobutyryl residue was chosen as it was already successfully used for the 2'-deoxy compound.[10] The tetraisobutyrylated derivative was prepared as intermediate according to Khorana[11] and the sugar protecting groups were selectively removed with 2 N aq. NaOH. With a $\tau_{1/2}$ of 115 min (25 % NH_4OH at 40°C) for 7-deaza-$\underline{N}^2$-isobutyrylguanosine, a similar rate of deprotection was observed as for the 2'-deoxy derivative. As 5'-OH-protecting group, the 4-methoxytrityl residue was introduced by the standard protocol,[12] resulting in **5**.



	R
a	$tBuMe_2Si$
b	$(iPr)_3Si$
c	$tBuPh_2Si$

Ogilvie and Usman [13,14] described RNA-building blocks (cyanoethylphosphoramidites) containing the t-butyldimethylsilyl group for 2'-OH-protection. Other authors demonstrated the use of this protecting group in the phosphonate approach.[15] Although silyl residues show several disadvantages - they can isomerize and are difficult to remove from oligomers, their selective deprotection with fluoride secures their current use in oligoribonucleotide synthesis. Three different trialkylsilyl chlorides ($tBuMe_2SiCl$, iPr_3SiCl, $tBuPh_2SiCl$) were used in the case of **5**, and either $AgNO_3$ or imidazole was employed as a catalyst. As Table 1 indicates, the highest 2'-selectivity was found in the case of triisopropylsilyl chloride and $AgNO_3$. In no case, formation of the 2',3'-bis-silylated compound was observed.

The position of the silyl residues was confirmed by ^1H-, ^{13}C-nmr- and ^1H-nmr NOE difference spectroscopy. Table 2 summarizes ^{13}C-nmr data. It can be seen that 2'-O-silylation leads to a downfield shift of the C-2' signal; 3'-O-silylation to a deshielding of C-3'. Furthermore, NOE experiments on compound (**6a**) show an NOE of 5% on 3'-OH after irradiation of H-4', while saturation of H-1' gives no effect.[16] On the other hand, irradiation of H-1' of the 3'-O-silylated derivative (**7a**) gives an NOE at 2'-OH (1%), while saturation of H-4' gives no effect.

Compounds (**6a**) and (**6b**) were converted into their methylphosphoramidites[18] [**4a**, **b** (^{31}P-nmr ($CDCl_3$): δ 151.9 and 150.2 ppm (**4a**); 153.0 and 149.6 ppm (**4b**))] as well as into phosphonates[19] [**3a**, **b** (^{31}P-nmr (DMSO-d_6): δ 2.20 ppm (**3a**) and 2.35 ppm (**3b**))].
As phosphonates are easier to handle than phosphoramidites and are highly stable against oxidation, we decided to use compounds (**3a**, **b**) in automated solid-phase synthesis. Two hexamers, $(GC)_3$ (**8**) and $(c^7GC)_3$ (**9**), were synthesized on an Applied Biosystems 380 B synthesizer. The cycle times for detritylation, coupling, and activation followed the protocol of Stawinski et al.[20] The 5'-O-detritylated oligomers were removed from solid support and deprotected at the base with ammonia/EtOH (3 : 1) at 55°C for 16 h. 2'-O-Deprotection was carried out with 1 M Bu_4NF in THF over 24 h at room temperature. After

desalting (Sephadex G25 F column; Pharmacia, Germany) under sterile conditions with 0.05 M ammonium acetate, 20% polyacrylamide gel electrophoresis (25 cm x 16 cm x 1.5 mm) under denaturating conditions was used for purification.[21] After elution from the gel with 0.1 M ammonium acetate, the oligomers were again desalted on a Sephadex column.

Table 1. Formation of 2'- and 3'-Isomers upon Silylation of **5**.

Silylating Agent[a)] [mmol]	Catalyst [mmol]		Yield of Isomers[b)] [%] (R_f)[c)]		Reaction Time[a)] [h]
	$AgNO_3$	imidazole	2'-Isomer	3'-Isomer	
$tBuPh_2SiCl$					
2.0	2.0	-	42 (0.65)	30 (0.55)	30
1.5	-	2.0	38	46	30
$tBuMe_2SiCl$					
1.5	1.5	-	56 (0.75)	21 (0.7)	24
1.5	-	1.5	25	63	19
iPr_3SiCl					
2.5	2.5	-	63 (0.7)	16 (0.6)	48
2.5	-	2.5	54	29	48

a) In all the cases, THF was used as a solvent; the reaction was started with 0.4 mmol of **5** and 1.0 eq. of silylating agent (1.5 eq. in the case of iPr_3SiCl) and after 12 h (24 h) the reminder was added to the total amount. b) Individual isomers were obtained by silica gel column chromatography (silica gel 60 H, Merck) using CH_2Cl_2/light petroleum ether /EtOAc, 1:1:1 as the eluent. c) Tlc on silica gel glass plates (Sil G-25 UV_{254}, Merck), solvent: CH_2Cl_2/light petroleum ether/EtOAc, 1:1:1.

Table 2. ^{13}C-Nmr Chemical Shifts of 7-Deazaguanosine Derivatives[a)]

compd.[b)]	C-4	C-2	C-7a	C-6	C-5	C-4a	CHiBu	CH_3iBu	CO
3a	156.6	148.8	147.2	118.8	103.7	104.0	34.9	18.9	180.1
3b	156.7	148.8	147.2	118.8	103.9	104.1	34.9	18.9	180.1
5[c)]	156.7	148.2	147.0	119.7	103.1	104.3	34.8	19.0	180.1
6a[d)]	156.6	148.4	146.9	118.9	103.4	104.0	34.7	18.8	180.0
7a[d)]	156.6	148.4	146.9	119.3	103.2	104.3	34.7	18.9	180.0
6b	156.7	148.6	147.0	119.0	103.7	104.2	34.8	18.9	180.1
7b	156.7	148.5	147.0	119.3	103.3	104.4	34.8	19.0	180.1
6c	156.7	148.4	146.9	119.0	103.5	104.3	34.8	18.9	180.1
7c	156.7	148.5	147.0	119.4	103.1	104.4	34.8	19.0	180.1

	C-1'	C-4'	C-2'	C-3'	C-5'	OCH_3	<u>C</u>-MMTr	$Me_3\underline{C}$-Si/$Me_2\underline{CH}$-Si
3a	84.9	83.7	75.0	73.2	63.9	55.1	86.1	17.7
3b	85.2	83.3	74.8	73.6	63.7	55.1	86.2	11.7
5	86.7	82.8	73.7	70.5	64.2	55.1	85.9	-
6a[d)]	85.6	83.9	76.1	70.7	64.0	55.0	86.0	17.7
7a[d)]	85.9	83.4	72.9[e)]	72.6[e)]	63.6	55.0	86.1	18.0
6b	86.1	84.1	75.9	71.1	64.0	55.2	86.1	11.8
7b	86.1	84.0	73.4[e)]	72.5[e)]	63.7	55.1	86.2	11.8
6c	86.0	84.4	76.4	70.7	63.9	55.2	86.0	18.8
7c	85.9	83.8	73.3[e)]	73.1[e)]	63.5	55.1	85.9	19.0

[a)] Spectra were measured in DMSO-$\underline{d}_6$ rel. to TMS. [b)] Systematic numbering. [c)] Assignment of aglycone signals according to the 2'-deoxyribonucleosides.[10,17] [d)] Assignment from gated-decoupled spectra. [e)] Tentative assignment.

The composition of the oligomers (8) and (9) was derived from enzymatic digestion using snake venom phosphodiesterase followed by alkaline phosphatase and analysis by reverse phase RP-18 hplc (Figure). Quantification was made on the basis of peak areas and the extinction coefficients of the nucleosides at 260 nm and shows equivalent amounts of G and C for **8**, and c^7G and C for **9**, respectively. The identity of compound (**1**) was proved by addition of synthetic **1**. The successful incorporation of c^7G (**1**) into an oligoribonucleotide opens a wide range of experiments to elucidate changes in secondary and tertiary structure caused by replacement of nitrogen-7 by carbon as well as to study the interaction with RNA-processing enzymes or other proteins.

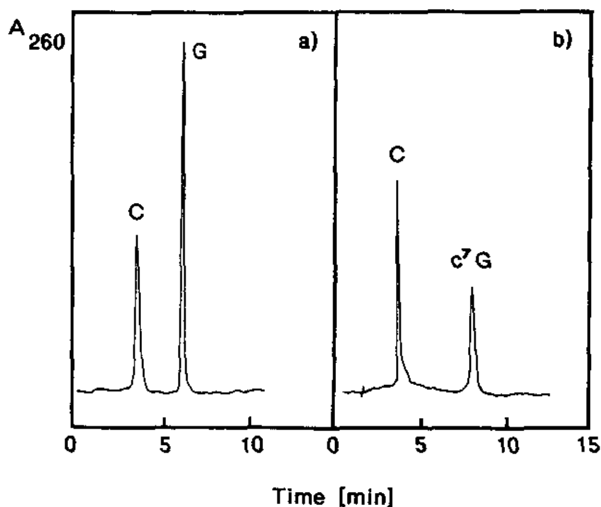


Figure. Hplc profiles of enzymatically hydrolyzed hexamers a) **8** and b) **9** carried out on RP-18 column (LiChrosorp (7 µm), 250 x 4.9 mm) using 5% MeCN in 0.1 N ammonium acetate; digestion was performed with snake venom phosphodiesterase followed by alkaline phosphatase; retention times were 3.6 min (cytidine), 6.3 min (guanosine) and 7.5 min (7-deazaguanosine).

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